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Qualitative Functional Group Analysis of Gas Chromatographic Effluents

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▶ The identification of components separated by gas chromatography cannot be accomplished with reasonable certainty by relying on retention data alone, particularly when the mixture is heterofunctional. A method employing functional group classification reagents has been developed which, when used with retention volume data from the corresponding chromatogram, gives positive identification of the components of a mixture. The method is simple and requires little extra time and no expensive supplementary instrumentation. The technique also frequently provides data to show whether a chromatographic peak consists of one or more components.

THERE is little doubt that gas chromatography has become one of the most elegant methods of analysis ever devised. But for the investigator who must deal with entirely unknown mixtures of compounds, the use of retention volume data alone for the identification of components separated by gas chromatography is a serious limitation. In many fields of analysis the mixtures frequently encountered contain a number of heterofunctional or isomeric components, and some of the components of such mixtures have the same, or nearly the same, retention volumes, even on different column materials. Although the analysis ultimately might be accomplished by finding suitable liquid phases to effect resolution and identification, the large number of liquid phases to be studied makes this approach time-consuming and unrewarding. Although the problem has been solved in some instances by collecting the eluted peaks and employing infrared spectrophotometry (*1, 2, 6, 8, 9, 11*) or mass spectrometry (*4, 5, 7*) for subsequent identification, the supplementary instrumentation is very expensive, and the procedures for collecting eluted chromatographic peaks are cumbersome and are not always reliable. Many laboratories do not have such instrumentation available and must rely on gas chromatography alone.

The failure of the gas chromatographic method to provide complete qualitative analysis is due primarily to the inability of the gas chromatograph to determine or distinguish organic functionality (*10*). If the functionality can be established, the identity of a particular compound can then be determined from its retention volume. This investigation was undertaken to develop the systematic use of functional group classification reagents to provide a direct, rapid, and inexpensive method of qualitative gas chromatographic analysis.

EXPERIMENTAL

Apparatus and Procedure. Conventional packed column–type gas chromatography apparatus with a thermal conductivity detector was used throughout this study. The functional group classification of eluted chromatographic peaks was accomplished by means of a stream-splitting device, shown in Figure 1, which was attached to the exit tube from the thermal conductivity cell. The splitter is constructed of a piece of 0.25-inch outside diameter stainless steel tubing, with a rubber serum cap with five hypodermic needles of the same gage inserted through the cap and into the open end of the stainless tubing. The chromatographic effluent is divided thereby into five equal streams, each of which is allowed to bubble through a vial containing an appropriate classification reagent.

The number of individual effluent streams in the splitter, and the number of reagents employed, will depend, of course, on the number of functional group–type compounds expected to be found in the mixture. A five-way split of the chromatographic effluent, however, was found most convenient. When a larger number of reagents was used, an even and continuous flow in all the vials could not be obtained and handling became rather cumbersome. It was generally found more convenient, if more reagents were required, to run a second sample with another set of reagents. Another factor which affects the choice of the number of functional group tests to be performed at one time is the sensitivity of the reagent, and therefore it is undesirable to decrease excessively the amount of component bubbling through the reagent.

As each chromatographic peak passes, it is split and allowed to pass through a set of the various reagents. To make a rapid change in the stream splitter and reagent vials, when successive peaks are closely adjacent, the exit tube is provided with a three-way stopcock so that as soon as one peak has passed, the stopcock may be turned and the second peak passes through a second splitter. While the second peak is passing, a new set of reagent vials may be positioned to receive the next succeeding peak.

Reagents. The reagents employed to test for the functional group classes are summarized in Table I. The sensitivities of the various reagents were all determined experimentally. Each of the classification reagents was initially selected if 0.5 mg. of a pure component would give a positive test. However, this does not represent the limit of sensitivity since in many instances the reagent will detect smaller amounts. The approximate minimum amount that could be detected by a given reagent was determined by injecting a known amount of a typical component onto the column, and after splitting, observing the response of the reagent. The values for minimum detectable amounts given in the table correspond to the estimated amount in the effluent stream passing through the reagent and represent approximately one fifth of the amount of sample injected on the column. Since the smallest sample which could be conveniently measured was 0.1 μl., the maximum sensitivity of any of the reagents was taken as 20 μg. Some of the reagents are actually even more sensitive as indicated by the strong response of the reagent at the 20-μg. level.

The sensitivity tests were made with appropriately chosen compounds containing two or three carbon atoms. The sensitivity of the reagents tends to decrease somewhat with increasing molecular weight of a component. All the reagents were checked for response over a fairly wide range of carbon number within each homologous series and the response was generally found to be adequate. The compounds tested are also given in the table.

The usual cautions must be observed, of course, in the general application and interpretation of the color tests because of the variations in sensitivity, unusual responses of the reagents, etc. In the selection and application of the various reagents, the worker is advised to consult freely with standard references on qualitative functional group reagents (*3, 12*).

In the studies reported here, nine functional groups were considered: alcohols, aldehydes, ketones, esters, unsaturated aliphatic and aromatic hydrocarbons, amines, alkyl halides,

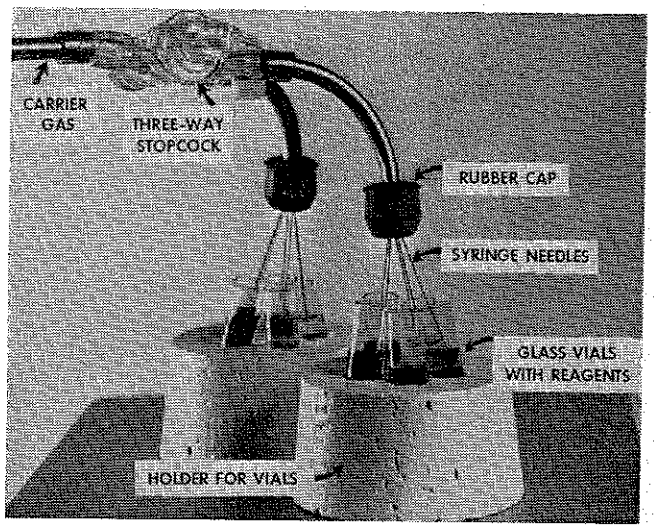

Figure 1. Stream-splitting device for functional group testing of eluted peaks

sulfur compounds, and nitriles. A description of the preparation of each of the reagents used in the test vials follows. The vials used were 3.5 cm. long × 1.0 cm. in diameter. Details of the preparation of the reagent solutions may be found in the references cited.

ALCOHOLS. Nitrochromic Acid (*3*): 10 drops (ca. 0.5 ml.) of 7.5*N* HNO_3 plus 1 drop of 1% $K_2Cr_2O_7$. Turns from bright yellow to blue-gray. Good for primary and secondary alcohols.

Ceric Nitrate (*3, 12*): 5 drops of reagent plus 5 drops of H_2O. Yellow to amber. Good for all aliphatic alcohols.

ALDEHYDES. Dinitrophenylhydrazine (*3, 12*): 10 drops. Yellow or orange precipitate.

Schiff's Reagent (*3, 12*): must be freshly prepared, 10 drops. Colorless to pink or purple.

KETONES. Dinitrophenylhydrazine (*3, 12*): 10 drops. Yellow or orange precipitate.

ESTERS. Ferric Hydroxamate (*3*): 10 drops of 1*N* NH_2OH.HCl in methanol plus 3 to 4 drops of 2*N* alcoholic KOH or until solution turns blue. After passing sample through solution add 5 to 6 drops of 2*N* HCl until solution is clear and colorless. Add 1 to 2 drops of 10% $FeCl_3$. Colorless to red.

ALKYL HALIDES. Alcoholic $AgNO_3$ (*12*): 10 drops of 2% alcoholic $AgNO_3$. White precipitate.

Mercurous Nitrate: 10 drops of a 7.5*N* HNO_3–5% mercurous nitrate solution. Iodides, yellow to orange precipitate; chlorides, white precipitate; bromides, white or gray precipitate.

AMINES. Benzenesulfonyl Chloride (Hinsberg test) (*3, 12*): 5 drops of pyridine, 1 drop of 5% NaOH. After sample passes, add 1 to 2 drops of benzenesulfonyl chloride. Colorless to yellow for primary or secondary aliphatic amines. Tertiary amines give a rose to deep purple color.

Sodium Nitroprusside (Rimini and Simon test for primary and secondary amines) (*3*): 10 drops of H_2O plus 2 drops of acetone plus 1 drop of 1% sodium nitroprusside. Primary amine gives red color. Add 1 to 2 drops of acetaldehyde. Secondary amine gives blue color. Omitting acetone permits test for secondary amine directly.

ALKYL NITRILES. Ferric Hydroxamate (*3*): 10 drops of 1*N* NH_2OH. HCl in propylene glycol plus 2 drops of 1*N* KOH in propylene glycol. After sample passes, heat to boiling and cool. Solution is clear and colorless. Add 1 to 2 drops of 10% $FeCl_3$. Red-wine color is positive test.

MERCAPTANS. Alcoholic Silver Nitrate (*12*): 10 drops of 2% alcoholic $AgNO_3$ gives white precipitate. (H_2S gives black precipitate.)

Lead Acetate (*3*): 10 drops of saturated alcoholic PbOAc gives yellow precipitate. (H_2S gives black precipitate.)

Isatin (*3*): 10 drops of 1% isatin in concentrated H_2SO_4 gives green color.

Sodium Nitroprusside: 10 drops of 95% ethyl alcohol plus 2 drops of a 5% KCN–1% NaOH solution. Two to three minutes after sample passes, add 5 drops of 1% sodium nitroprusside solution. Red color results.

ALKYL SULFIDES. Sodium Nitroprusside: Same as test for Sodium Nitroprusside under Mercaptans above.

ALKYL DISULFIDES. Isatin: Same as test for Isatin under Mercaptans above.

Sodium Nitroprusside: Same as test for Sodium Nitroprusside under Mercaptans above.

AROMATIC NUCLEUS AND ALIPHATIC UNSATURATION. Formaldehyde–sulfuric acid(LeRosen test) (*3*): 10 drops of concentrated H_2SO_4 plus 1 drop of 37% HCHO gives wine color.

The reagents used, of course, will depend on the types of compounds expected and on the desired level of response of the reagent. The potassium dichromate–nitric acid (nitrochromic acid) test was found most suitable for alcohols. This reagent proved to be more sensitive than the more conventional ceric nitrate test. However, the nitrochromic acid reagent fails to respond to tertiary alcohols, in which case, ceric nitrate reagent then may be used.

In some cases, the response of two or more reagents must be considered in combination. For example, since both aldehydes and ketones give a positive reaction with 2,4-dinitrophenylhydrazine, Schiff's reagent was used to distinguish aldehydes from ketones. Mercaptans, sulfides, and disulfides all give a positive test with sodium nitro-

Table I. Functional Group Classification Tests

Compound Type	Reagent	Type of Positive Test	Minimum Detectable Amt., μg.	Compounds Tested
Alcohols	$K_2Cr_2O_7$–HNO_3	Blue color	20	C_1–C_8
	Ceric nitrate	Amber color	100	C_1–C_8
Aldehydes	2,4-DNP	Yellow ppt.	20	C_1–C_6
	Schiff's	Pink color	50	C_1–C_6
Ketones	2,4-DNP	Yellow ppt.	20	C_3–C_8 (methyl ketones)
Esters	Ferric hydroxamate	Red color	40	C_1–C_5 acetates
Mercaptans	Sodium nitroprusside	Red color	50	C_1–C_9
	Isatin	Green color	100	C_1–C_9
	$Pb(OAc)_2$	Yellow ppt.	100	C_1–C_9
Sulfides	Sodium nitroprusside	Red color	50	C_2–C_{12}
Disulfides	Sodium nitroprusside	Red color	50	C_2–C_6
	Isatin	Green color	100	C_2–C_6
Amines	Hinsberg	Orange color	100	C_1–C_4
	Sodium nitroprusside	Red color, 1°	50	C_1–C_4
		Blue color, 2°		Diethyl and diamyl
Nitriles	Ferric hydroxamate–propylene glycol	Red color	40	C_2–C_5
Aromatics	HCHO-H_2SO_4	Red-wine color	20	ϕH–ϕC_4
Aliphatic unsaturation	HCHO-H_2SO_4	Red-wine color	40	C_2H_8
Alkyl halide	Alc. $AgNO_3$	White ppt.	20	C_1–C_5

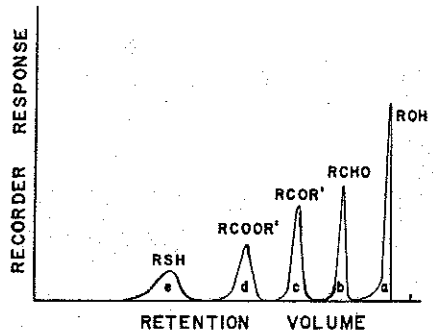

Figure 2. Chromatogram of five-component mixture

6-foot squalane column, 125° C.
Flow, 50 ml. per minute
Sample, 1.0 μl.

prusside. This reagent, therefore, may be used as a general test for the presence of these sulfur compounds. If one wishes to distinguish between mercaptans, sulfides, and disulfides, lead acetate may be used to detect mercaptans, and the isatin test may be used to distinguish between sulfides and disulfides.

The reaction of esters with hydroxylamine to form the corresponding hydroxamic acid proved successful for the classification of both esters and nitriles, but nitriles may be distinguished from esters since the development of the color for nitriles must take place in propylene glycol solution. In either case, the color reaction is not given immediately, as the effluent stream passes through the reagent, but must be subsequently developed by the addition of ferric chloride.

Similarly, the formaldehyde–sulfuric acid reagent is used to detect both aromatic compounds and aliphatic unsaturation. However, these types may be differentiated on the basis of retention time, once the presence of either type is established.

Primary and secondary amines can be detected by either the Hinsberg reaction or by the Rimini and Simon test with sodium nitroprusside. The sodium nitroprusside test for amines employs different reaction conditions than the sodium nitroprusside test for sulfur compounds. It is this difference in reaction conditions which permits the identification of an amine or a sulfur compound with the same reagent. The sodium nitroprusside reagent is preferable to benzenesulfonyl chloride used in the Hinsberg reaction since the color reaction immediately distinguishes between a primary and a secondary amine. On the other hand, tertiary amines may be distinguished from primary and secondary amines with benzenesulfonyl chloride if modified reaction conditions (*3*) are employed.

RESULTS AND DISCUSSION

The fact that certain members of different homologous series of compounds may have identical retention characteristics has prevented the optimum use of gas chromatography for qualitative analysis of heterologous mixtures isolated from natural products. Retention volume data alone, without a knowledge of compound functionality, do little more than indicate several possibilities for the identity of an individual compound of given retention volume. The results of this investigation have shown that the determination of component functionality with classification reagents can lead to direct qualitative identification of individual components of heterologous mixtures.

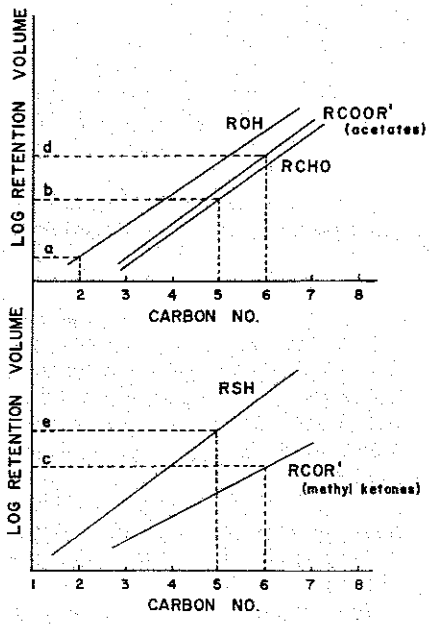

Figure 3. Graph showing identification of individual compounds corresponding to peaks shown in Figure 2

A chromatogram of a five-component mixture is shown in Figure 2. Each of the peaks, as it was eluted from the column, was passed through the various classification reagents and its functionality was determined by the resulting change in one of the reagents. The individual compounds were then identified by comparing the corrected retention volume of the eluted peak with the corresponding corrected retention volumes of the individual members of the appropriate homologous series. This may be accomplished most conveniently by a graphical procedure (*10*). As shown in Figure 3, a plot of the log of the retention volume *vs.* carbon number is prepared for each homologous series of which a member has been shown to be present by means of the functional group tests. Interpolation on this graph then permits identification of the various components as individual compounds. The alcohol was thus identified as ethyl alcohol, the aldehyde as pentanal, the ketone as 2-hexanone, the ester as butyl acetate, and the mercaptan as pentanethiol.

Frequently, in the analysis of unknown mixtures, neither the types of components nor the total number of components is known, and the chromatogram resulting from such a mixture may show peaks which result from two or more components having the same retention time. Without the aid of functional group classification reagents, this situation can be resolved only with difficulty, if at all. Ordinarily, several chromatograms would have to be obtained on different stationary phases. The situation can be resolved quite readily, however, by means of the functional group classification reagents. This may be illustrated by means of the chromatogram shown in Figure 4. The chromatogram appears to be that of a mixture having five components. Functional group tests showed, however, that the first peak, *a*, was due to an alcohol and a ketone; the second, *b*, a ketone and an ester; the third, *c*, a mercaptan; the fourth, *d*, an aldehyde, an alcohol, and an aromatic hydrocarbon; and the last, *e*, an aldehyde and an aromatic hydrocarbon. Thus, ten components were present rather than the five which were indicated by the chromatogram. The individual compounds were subsequently identified from the appropriate log retention volume *vs.* carbon number plots as shown in Figure 5.

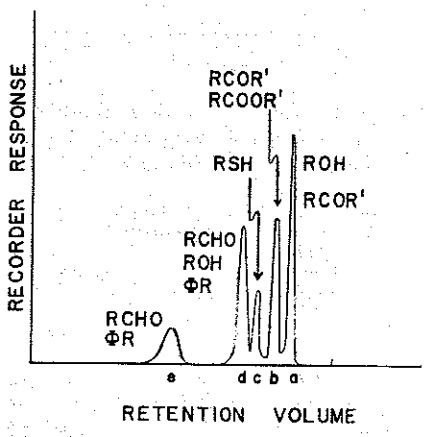

Figure 4. Chromatograms of unresolved 10-component mixture

6-foot squalane column, 125° C.
Flow, 50 ml. per minute
Sample, 2.0 μl.

Three situations arise in the use of log retention volume *vs.* carbon number plots which require further clarification. The first case arises when the

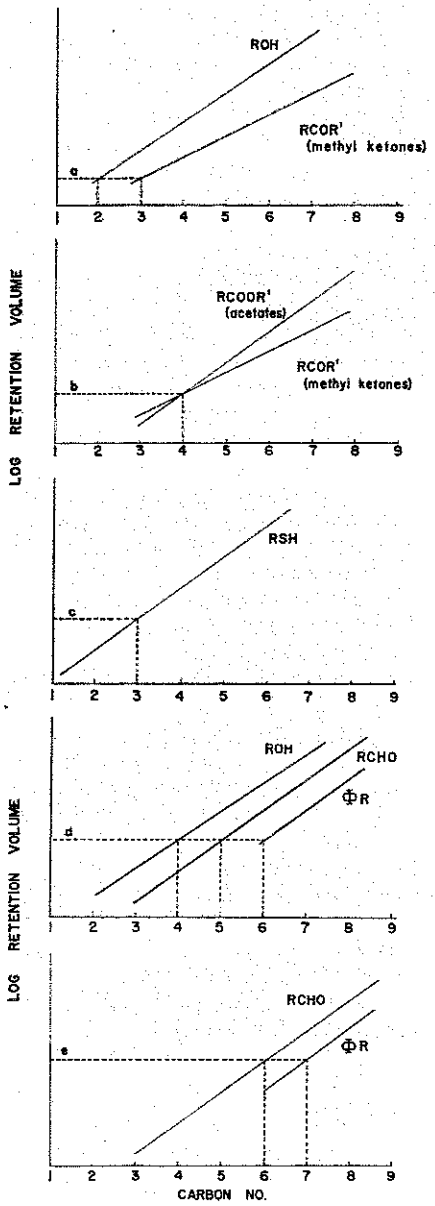

Figure 5. Graph showing identification of individual compounds corresponding to peaks in Figure 4

- *a.* Ethyl alcohol, 2-propanone
- *b.* Ethyl acetate, 2-butanone
- *c.* Propanethiol
- *d.* Butyl alcohol, pentanal, benzene
- *e.* Hexanal, toluene

same reagent is used to detect more than one type of functional class. For example, the LeRosen test is used to detect both aromatic and aliphatic unsaturation. Little difficulty arises, however, in identifying the individual components from retention volume once the compound has been classified as either aromatic or aliphatic unsaturated. This is illustrated in Figure 6 where the log retention volume *vs.* carbon number plots for both alkylbenzenes and olefins are shown. The log retention volume for a given compound will intersect the plot corresponding to an integral carbon number only for the appropriate compound. In this example, a compound having the retention volume shown must be toluene and could not be octene.

A second situation arises from the fact that the classification tests usually fail to distinguish among isomers within

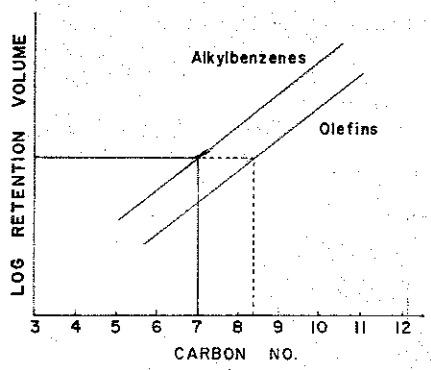

Figure 6. Graph showing identification of LeRosen positive peak as toluene

the functional group class. Fortunately, however, log retention volume *vs.* carbon number plots are valid for any given series of isomers within a functional class. Thus, one can obtain suitable plots for groupings such as normal alcohols, iso-alcohols, or secondary alcohols; methyl ketones or ethyl ketones; primary or secondary amines, etc. The application of this principle is illustrated in Figure 7. The log retention volume *vs.* carbon number plot is shown for both the normal alcohols and the isomeric alcohols. The graph indicates that the alcohol having the retention volume shown would have to be isobutyl alcohol, and could not be normal butyl alcohol nor normal propyl alcohol.

Finally, when two classification re-

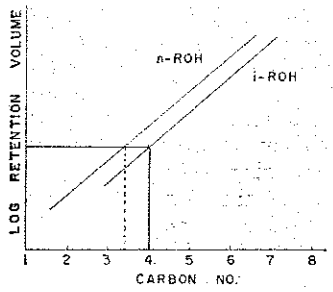

Figure 7. Graph showing identification of nitrochromic acid positive peak as isobutyl alcohol

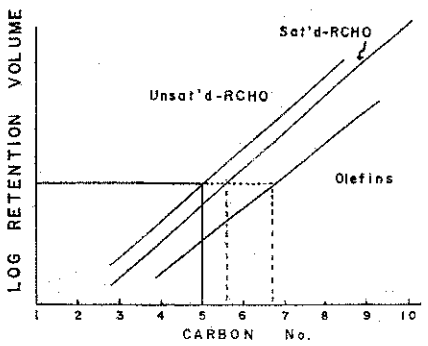

Figure 8. Graph showing identification of LeRosen and Schiff's positive peak as pentenal

agents respond for a given peak, the question arises whether the peak is due to two components or a bifunctional compound. For example, if positive results are obtained from the reaction of an eluent peak with both Schiff's and the LeRosen reagents, the possibility of whether the peak is due to an unsaturated aldehyde or a combination of an olefin and a saturated aldehyde must be considered. Figure 8 shows, however, from the plots for the corresponding saturated aldehydes, unsaturated aldehydes, and olefins, that the compound having the retention volume shown would have to be pentenal, the unsaturated five-carbon aldehyde, and could not be a mixture of an olefin and a saturated aldehyde.

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